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EXAMINER	
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ART UNIT	PAPER NUMBER
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/530,401	TANAKA ET AL.	
	Examiner	Art Unit	
	Maria Leavitt	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 October 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *04-07-2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claim Status. Claims 1-9 are pending. Applicant's election of the following species in the reply filed on 10/01/2007 is acknowledged: a blastocyst as recited in claim 5. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Upon further consideration, the examiner **withdraws** the species restriction requirement of 8-cell-stage embryos, morula and blastocysts, as recited in claim 5. A search of prior art would be overlapping, and thus there is no undue burden in doing a search for all the species together

The requirement is still deemed proper and is therefore made FINAL.

Therefore claims 1-9 are currently under examination to which the following grounds of rejection are applicable.

Information Disclosure Statement

The information disclosure statements filed on 04/07/2005 have been reviewed, and their references have been considered as shown by the Examiner's initials next to each citation on the attached copies.

The information disclosure statement filed on 04/07/2005 fails to comply with 37 C.F.R. § 1.98(a)(2), which requires a legible copy of each U.S. and foreign patent; each publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed. The following references were not considered for the reasons described below:

1. Reference A1 is incomplete in the absence of a legible copy,
2. Reference A2 is incomplete in the absence of a legible copy,
3. Reference A4 is incomplete in the absence of a legible copy, and
4. Reference A5 is incomplete in the absence of a legible copy.

All other documents in said Information Disclosure statement were considered as noted by the Examiner initials in the copy attached hereto.

Objection Drawings

The drawings are filed on 04-07-2005 are objected to under 37 CFR 1.83(a). The drawings must show every feature of the invention specified in the claims.

Fig. 3A-3D are photographs showing the malformation in appearance of chimeric mice obtained using an embryonic stem cell line 281 wherein FIG. 3A is identified as chimeric mice No. 581m, FIG. 3B is identified as chimeric mice No. 582m, FIG. 3C is identified as chimeric mice No. 584f, and FIG. 3D is identified as chimeric mice No. 580m. However, the pictures are too dark to visualize any the malformation of chimeric mice. Therefore, photographs showing the malformation of chimeric mice must be shown or the feature(s) canceled from the claim(s). No new matter should be entered.

Fig. 4A-3D are photographs showing the malformation in appearance of chimeric mice obtained using an embryonic stem cell line 344 wherein FIG. 4A is identified as chimeric mice No. 589m, FIG. 4B is identified as chimeric mice No. 587m, FIG. 4C is identified as chimeric mice No. 585f, and FIG. 3D is identified as chimeric mice No. 588m. However, the pictures are too dark to visualize any the malformation of chimeric mice. Therefore, photographs showing the malformation of chimeric mice must be shown or the feature(s) canceled from the claim(s). No new matter should be entered.

Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Rejections - 35 USC § 101- Lack of Utility

35 U.S. C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.
>
> Definitions.
>
> (From REVISED INTERIM UTILITY GUIDELINES TRANINING MATERIALS; repeated from http://www.uspto.gov/web/menu/utility.pdf)
>
> "Credible utility" – Where an applicant has specifically asserted that an invention has a particular utility, that assertion cannot simply be dismissed by Office personnel as being "wrong". Rather, Office personnel must determine if the assertion of utility is credible (i.e., whether the assertion of utility is believable to a person of ordinary skill in the art based on the

totality of evidence and reasoning provided). An assertion is credible unless (A) the logic underlying the assertion is seriously flawed, or (B) the facts upon which the assertion is based are inconsistent with the logic underlying the assertion. Credibility as used in this context refers to the reliability of the statement based on the logic and facts that are offered by the applicant to support the assertion of utility. A *credible* utility is assessed from the standpoint of whether a person of ordinary skill in the art would accept that the recited or disclosed invention is currently available for such use. For example, no perpetual motion machines would be considered to be currently available. However, nucleic acids could be used as probes, chromosome markers, or forensic or diagnostic markers. Therefore, the credibility of such an assertion would not be questioned, although such a use might fail the *specific* and *substantial* tests (see below).

"Specific utility" – A utility that is *specific* to the subject matter claimed. This contrasts with a *general* utility that would be applicable to the broad class of the invention. For example, a claim to a polynucleotide whose use is disclosed simply as a "gene probe" or "chromosome marker" would not be considered to be *specific* in the absence of a disclosure of a specific DNA target. Similarly, a general statement of diagnostic utility, such as a diagnosing an unspecified disease, would ordinarily be insufficient absent a disclosure of what condition can be diagnosed.

"Substantial utility" - a utility that defines a "real world" use. Utilities that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use are not substantial utilities. For example, both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a "substantial utility" define a "real world" context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a "real world" context of use in identifying potential candidates for preventive measures or further monitoring. On the other hand, the following are examples of situations that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use and, therefore, do not define "substantial utilities":

A. Basic research such as studying the properties of the claimed product itself or the mechanisms in which the material is involved.

B. A method of treating an unspecified disease or condition. (Note, this is in contrast to the general rule that treatments of specific diseases or conditions meet the criteria of 35 U.S.C. § 101.)

C. A method of assaying for or identifying a material that itself has no "specific and/or substantial utility".

D. A method of making a material that itself has no specific, substantial and credible utility.

E. A claim to an intermediate product for use in making a final product that has no specific, substantial and credible utility.

Note that "throw away" utilities do not meet the tests for a *specific* or *substantial* utility. For example, using transgenic mice as snake food is a utility that is neither specific (all mice could function as snake food) nor substantial (using a mouse costing tens of thousands of dollars to produce as snake food is not a "real world" context of use). Similarly, use of any protein as an animal food supplement or a shampoo ingredient are "throw away" utilities that would not pass muster as specific or substantial utilities under 35 U.S.C. §101. This analysis should, of course, be tempered by consideration of the context and nature of the invention. For example, if a transgenic mouse was generated with the specific provision of an enhanced nutrient profile, and disclosed for use as an animal food, then the test for specific and substantial *asserted* utility would be considered to be met.

"Well established utility" - a specific, substantial, and credible utility which is well known, immediately apparent, or implied by the specification's disclosure of the properties of a material, alone or taken with the knowledge of one skilled in the art. "Well established utility" does not encompass any "throw away" utility that one can dream up for an invention or a nonspecific utility that would apply to virtually every member of a general class of materials, such as proteins or DNA. If this were the case, any product or apparatus, including perpetual motion machines, would have a "well established utility" as landfill, an amusement device, a toy, or a paper weight; any carbon containing molecule would have a "well established utility" as a fuel since it can be burned; and any protein would have well established utility as a protein supplement for animal food. This is not the intention of the statute.

See also the MPEP § 2107-2107.02

Claims 1-9 are rejected under 35 U.S. C. 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well-established utility.

The claims are directed to a chimeric mouse having an endogenous Bradeion gene, the gene expression of which has been suppressed, and a cell derived from a mouse embryonic stem cell obtained from said chimeric mice. Moreover, the claims read on a "malformation" phenotype including cranial dysplasia, visual disorders, and generalized decreased growth as a result of suppressing expression of an endogenous Bradeion gene.

The specification teaches that chimeric mice produced and born comprise somatic cells and germ cells which consist of original-line-derived and introduced-embryonic-stem-cell-derived cells wherein the "the rate of the contribution (chimeric rate) of the embryonic stem cell to the tissues can be calculated based on proportional comparison of the body hair color of the strain from which the embryonic stem cell is derived and that of the original strain " (p. 11, lines 13-24). Moreover, chimeric mice are contemplated to "be useful as a model for disorders and/or diseases associated with cerebral neurons and relating to cell canceration" (p. 5, lines 10-15). Additionally, the chimeric mice, wherein the expression of the endogenous Bradeion gene has been suppressed, exhibit hypoplasia of the cerebral nervous system and malformation. Thus, the chimeric mice of the present invention may be used to evaluate various treatments in diseases associated with hypoplasia in the cerebral nervous system and malformations (p. 4, lines 10-20).

The specification does not teach any examples of chimeric heterozygous and/or homozygous mice associating the disruption in a Bradeion gene with any cranial dysplasia, visual disorders, and generalized decreased growth. The specification merely teaches in Fig. 3A-3D and Fig. 4A-4D that the chimeric mouse exhibits malformation which includes cranial dysplasia, visual disorders, and generalized decreased growth wherein the chimeric rate is 90% or more and less than 98% (p.3, lines 17-24; p. 19, lines 15-20). It is noticed that the instant

claims broadly embrace chimeric mice wherein only one somatic cell may have been disrupted for endogenous expression of a Bradeion gene resulting in any percentages of chimerism other than 90 to 98%. Further, the specification contemplates using the chimeric mice as animals for genetic breeding associated with abnormalities in the cerebral nervous system (p. 19, lines 29-30). Indeed, there is no disclosure of any germline transmission. Moreover, the specification fails to provide specific examples of any cerebral nervous system and malformations disorders modulated by the Bradeion and any evidence that the claimed transgenic mouse would respond differently that a wild-type mouse in any of these tests, in any useful way.

Thus there is no supporting evidence for a correlation between any chimeric mouse exhibiting a dysfunctional Bradeion and a phenotype exhibiting cranial dysplasia, visual disorders, and generalized decreased growth. The specification teaches at page 19 that chimeric mice derived from mouse embryo wherein a mouse embryonic stem cell was introduced comprising an endogenous Bradeion gene, whose expression was suppressed, have chimeric rates between 90%-98%. Applicants are silent about any observed phenotype for any other percentage of chimerism other than within 90%-98%. It is noticed that only claim 7 recites the limitation of a defined phenotype for the chimeric mice, therefore claims 1-6, 8 and 9 lack utility as there is not correlation of the chimeric mice with any useful phenotype. It is also noticed that the claimed phenotypes may be associated with disruption of other genes in addition to Bradeion. For example, the art at the time of filing teaches that transgenic mice mutants for specific disruption of the beta-amyloid precursor protein (APP) exhibit cortical dysplasias characterized by focal ectopic neuroblasts which is not a phenotype expected for the disruption of said gene (Herms et al., The EMBO Journal, 2004, pp. 4106-4115, Abstract). Conversely, disruption of

specific genes may exhibit pleiotropic roles in many different cell types and tissues, for example, expression of Bradeion is associated with tumor-specific colorectal cancer, in addition to the instantly claimed phenotype (Tanaka et al., Biochem Biophys Res Commun. 2001 pp:547-53; p. 552, col. 1) .Thus, the specification contemplates uses for the claimed cells and transgenic mice to identify the functions of the disrupted Bradeion gene. However, this is not considered a "substantial utility" or a "specific utility" since such activity constitutes using the invention as an object of research in order to determine a use for the invention, and does not meet the requirement for a specific and substantial utility. *Brenner v. Mason,* 148 USPQ 689 (US SupCt. 1966).

At the time of the claimed invention, one skilled in the art would not have found such utilities evident, because the art is devoid of any teaching for a role or function for the Bradeion gene in modulation of cranial dysplasia, visual disorders, and generalized decreased growth. The art discloses that Bradeion has two distinct transcripts of approximately 2.2 and 1.7 kb length (alpha and beta, respectively) mainly in brain and slightly in heart, and no expression in any fetal organs. Ectopic expression of normal Bradeion alpha and beta transcripts were confirmed both in patients' tumor samples and *in vitro* cultured human cancer cell lines (Tanaka et al., Biochem Biophys Res Commun. 2001 pp:547-53; Abstract). Moreover, the art discloses that Bradeion is a novel human septin protein, which is specifically expressed, in colorectal cancer and malignant melanoma (Tanaka et al., Cancer Gene Ther. 2002: 483-8, Abstract). Additionally, both proteins encoded by the alpha and beta transcript can induce apoptosis in the cultured-undifferentiated human nerve cells (JP 2000139470, Date of publication 05/23/2000). However, the art is silent about any association of the Bradeion gene and modulation of cranial dysplasia, visual disorders,

and generalized decreased growth as embraced by the current invention.

Additionally, the specification fails to provide any correlation between the functions or role of the Bradeion disrupted gene regarding prevention, amelioration or correction of dysfunctions associated with Bradeion. Further, no data or specific statistics are disclosed regarding the significance of modulating the Bradeion in the chimeric mice and any data related to cranial dysplasia, visual disorders, and generalized decreased growth. Moreover, as it will be discussed below, Gerlai R. (Trends in Neurosci. 19 (5): 177-181, 1996) anticipates doubts as to whether the phenotype of a specific knock-out mouse is characteristic of all knock-out mice carrying a disruption in the same gene and factors such as genetic background and contributions of genes linked to the Bradeion gene should be considered. Omission of these factors may lead to misinterpretation of results. Thus in order to determine a specific utility for the mice, one skilled in the art would need to perform further research upon the claimed transgenic mice to conclude the correlation between the Bradeion chimeric mice displaying disruption of the Bradeion and its association with malformation including cranial dysplasia, visual disorders, and generalized decreased growth.

As set fort in the utility guidelines above, a general statement of diagnostic utility, such as using the instant mice to merely point out association with any malformation including cranial dysplasia, visual disorders, and generalized decreased growth is not considered 'substantial utility". Note that it was scientifically well known to knock-out a gene to determine its function (Capecchi, Science 244:1288-1292, 1989). However, scientific "utility" is not the same as "patentable utility" or a "well established utility". The MPEP and utility guidelines clearly set fort that a "well established utility" must be specific, substantial and credible. At the time of

filing, knockout mice were used for further research in the art. However, further research does not rise to the level of a "well established utility" because such utility is not specific and substantial.

The utility guidelines specifically states that further research is not a "substantial " utility:

The following are examples of situations that require or constitute carrying our further research to identify or reasonably confirm a "real world" context of use an, therefore, do not define " substantial utility":

A. Basic research such as studying the properties of the claimed product itself or the mechanisms in which the material is involved.

In the instant case, the simple recitation that the "an endogenous Bradeion gene, the gene expression of which has been suppressed" which transgenic mice exhibit malformation, does not provide a specific or substantial use for the claimed mice. Furthermore, the lack of specific teaching in the specification with regard to the Bradeion gene provides evidence that further study and experimentation would be required in order to determine the association of a disruption of the Bradeion with any unspecific malformation condition. The evidence of record has not provided any other utilities for the transgenic mice encompassed by the claims that are substantial and specific. The specification essentially gives an invitation to experiment wherein the artisan is invited to elaborate a functional use for the transgenic mouse encompassed by the claims. Though the human Bradeion has been cloned and its functionality associated with growth and tumorigenesis of colorectal cancer, its expression, distribution and physiologic function other than as a marker in colorectal cancer and malignant melanoma, has not been clearly defined. Hence using the chimeric mice to evaluate various treatments in diseases

associated with hypoplasia in the cerebral nervous system and malformations would require further research, which is not considered a "substantial utility" or "specific utility".

Overall, the chimeric mice and cells claimed do not have a "well established utility" because using the mouse for further research to determine the correlation between the functions or role of the Bradeion disrupted gene regarding malfunctions including cranial dysplasia, visual disorders, and generalized decreased growth doest not constitute a "substantial utility" or "specific utility". Further, the instant specification has not provided any other utilities for the transgenic mice encompassed by the claims that are substantial and specific. Accordingly, one of skilled in the art would not find the asserted utility of the transgenic mouse and cells, as claimed, to be specific and substantial.

Claim Rejections - 35 USC § 112- First paragraph- Enablement

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-9 are rejected under 35 U.S.C. 112, first paragraph.

The specification does not reasonably provide enablement for claims directed to a chimeric mouse generated from a mouse embryo, wherein the mouse embryo has a mouse embryonic stem cell introduced therein, said chimeric mice has a genomic DNA containing an endogenous Bradeion gene, the gene expression of which has been suppressed and a cell derived from said transgenic mice

The specification does not enable any person skilled in the art to which it pertains or with which it is most nearly connected, to use the invention commensurate in scope with this claim. Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims

The present invention is directed to generic transgenic mouse wherein an endogenous Bradeion gene expression has been suppressed. Claim 7 further limits the claimed transgenic mice to a phenotype that exhibits cranial dysplasia, visual disorders, and generalized decreased growth. The claims broadly read into any level of suppression of the Bradeion gene (e.g., 10%, 35% and others) able to induce the claimed phenotype. Moreover, the claims broadly encompass any targeting of an endogenous Bradeion gene so as to reduce or prevent its expression and biological functionality.

The specification teaches in Example 3 the use of stem cell lines 281 and 344 each injected into blastocysts of C57BL/6 mice by microinjection, which are then transferred into the oviducts of pseudopregnant female mice for the embryos to generate and develop into mice (p. 18 bridging to p.19 lines 1-15). Moreover, the specification teaches on Fig. 3A-3D and Fig. 4A-4D chimeric mice generated from embryonic stem cell lines 281 and 344, respectively, exhibiting a malformation which includes cranial dysplasia, visual disorders, and generalized

decreased growth wherein the chimeric rate is 90% or more and less than 98% (p.3, lines 17-24; p. 19, lines 15-20). In relation to targeting the endogenous mouse Bradeion gene, the specification teaches construction of target vectors wherein the cloned Bradeion gene has been mutated and each flanking region of said gene contain domains homologous to the 5' side and 3' side regions of the endogenous mouse Bradeion gene. Additionally, genetic markers for gene resistance and for body hair color can be utilized for positive selection of chimeric embryos and chimeric rate, respectively (pp. 7-8). Further, the specification contemplates using the chimeric mice as animals for genetic breeding associated with abnormalities in the cerebral nervous system (p. 19, lines 29-30). However, the specification fails to provide specific examples of any cerebral nervous system and malformations disorders modulated by any level of reduced expression of Bradeion and any evidence that the claimed transgenic mice would respond differently that a wild-type mouse in any of these malformations, in any useful way. Moreover, the specification fails to teach how targeting an endogenous Bradeion gene by any genetic mechanism of gene suppression results in reduced Bradeion activity.

The claims when given the broadest reasonable interpretation encompass a genus of unspecified variants for phenotypes associated with a Bradeion chimeric mouse, using any inbred strains of mice to produce the transgenic mouse. Only claim 7 defines a phenotype for the chimeric mice characterized by prevention, amelioration or correction of associated with hypoplasia in the cerebral nervous system (e.g., cranial dysplasia, visual disorders, and generalized decreased growth), therefore claims 1-6, 8 and 9 broadly embrace chimeric mice associated with a genus of unspecified phenotypes. Specific considerations for generation of a transgenic mouse such as the fate of the targeting vector in relation to a particular disruption, the

effect of the laboratory environment in the expression of an observed phenotype and the different contributions of the inbred strains of mice commonly used to make chimeric mice have to be addressed for phenotypes associated with chimeric Bradeion mice. The detail of the disclosure provided by the Applicant, in view of the prior Art, must encompass a wide area of knowledge to enable one of ordinary skill in the art at the time of the invention to practice the invention without undue experimentation. However, as it will be discussed below this undue experimentation has not been overcame by the as-filed application. Though Applicant's specification discloses the use of chimeric mice wherein expression of Bradeion has been reduced and/or prevented resulting in chimeric rates of 90% to 98% exhibiting a phenotype associated with hypoplasia in the cerebral nervous system and malformation dysfunctions e.g., cranial dysplasia, visual disorders, and generalized decreased growth, the broad aspects of a genus of unspecified variants for phenotypes associated with a chimeric Bradeion mice, using any inbred strains of mice to produce the transgenic mouse is not reasonably enable for the full scope embraced by the claims.

Regarding the claimed invention drawn to the phenotype of a transgenic chimeric mouse expressing a specific phenotype characteristic of all the chimeric mice carrying a disruption in the same gene, the art teaches that the resulting phenotype of a knockout mouse is unpredictable. First, as disclosed in the specification (p. 7, lines 7-29) if the targeting vector includes a heterologous gene, e.g., a marker gene such as a neomycin resistance gene, transcription of the heterologous gene may affect expression of the nearby genes. In that case, one cannot determine whether an observed phenotype is due to the inactivation of the targeted gene, e.g., Bradeion, or to alteration in expression of a nearby gene due to the presence of the heterologous gene in the

target vector. Holschneider et al., (Int J. Devl. Neuroscience 18:615-618, 2000) states: "knocking out or insertion of a single gene may result in no phenotypic change. This may be the case, in particular, if there exits gene redundancy mechanisms whose presence may prevent abnormal phenotypes from becoming masked. Conversely, single genes are often essential in a number of different behaviors and physiologic processes, e.g., expression of Bradeion is associated with tumor-specific colorectal cancer (Tanaka et al., Biochem Biophys Res Commun. 2001 pp:547-53; p. 552, col. 1). Hence, ablation of an individual gene may prove so drastic as to be lethal, or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interaction of the various new physiologic changes or behaviors" (Holschneider et al., p. 615, columns 1-2). Similarly, the author discusses various factors that contribute to the resulting phenotype of transgenic mice, including compensatory systems, which may be due to the differential expression of another gene, which may be regulated by the downstream product of the ablated gene, as well as the variability in phenotypic characterization due to particular mouse strains (see, p. 616, column 1). Further, Leonard (Immunol Rev. 1995 Dec;148:97-114) discloses mice with a disruption in the Y_c gene that was intended to be a model for X-linked severe combined immunodeficiency (XSCID), but display a variety of unexpected traits (Abstract). These knockout mice were expected to have thymocytes with decreased proliferation in response to stimulation with antibodies, but the thymocytes proliferated normally (page 105, line 7). Likewise, Griffiths (Microscopy Research and Technique, 41:344-358, 1998) taught that, despite a known role for the PLP gene based on spontaneous mutations in the gene, the chimeric mouse failed to display any of the expected phenotypes (page 350, last paragraph). Indeed, the as-filed specification discloses chimerics for Bradeion having a malformation

characterized by dysplasia; however, the art also teaches that cerebral dysplasia is the results of other disrupted genes in addition to the Bradeion gene such as chimeric mice for the a-amyloid precursor protein (APP) which exhibit cortical dysplasias characterized by focal ectopic neuroblasts (Herms et al., The EMBO Journal, 2004, pp. 4106-4115, Abstract). Thus the phenotype claimed for the disrupted Bradeion gene of the instant chimeric mice may result from other disrupted genes. Even post filing art cautions about interpretation of phenotypes in knock out mice that could be mistaken for a direct result of target gene manipulation rather than "a congenic footprint". For example, Schalkwyk et al., (Genes Brain Behav, 6:299-303, 2007; Abstract) demonstrated a "congenic footprint," a remaining fragment of the flanking stem cell-derived chromosome that causes differences in gene expression in cholecystokynin 2 knockout. Thus, the art at the time of filing and post filing art teach that the resulting phenotype of a knockout was considered unpredictable.

Additionally, the claimed invention broadly embraces disruption of the endogenous Bradeion gene by any genetic mechanisms of gene suppression including targeting the coding sequence of the Bradeion gene with an antisense DNA and/or RNA probe. The as filed specification only provides sufficient guidance for target vectors wherein the cloned Bradeion gene has been mutated and each flanking region of said gene contain domains homologous to the 5' side and 3' side regions of the endogenous mouse Bradeion gene for homologous recombination. The resulting chimeric mice comprise either a heterozygous or a homozygous disrupted Bradeion gene exhibiting a phenotype characterized by any malformation.

Regarding the claimed invention drawn to the observed behavioral phenotype, the laboratory environment in which the mice are kept and studied may have an effect on observed

behavioral phenotypes. The difference in degree of many behavioral phenotypes between mice of different genetic backgrounds can differ between laboratories (Crabbe et al., Science 284: 1670-1672, 1999). Thus, the phenotype of the Bradeion knock out mice relative to the "wild-type control" mice e.g., chimeric rate (Specifications p. 8, lines 15-29) observed by Applicant in their laboratory, may not be reproducible if the relative phenotype were assessed by another skilled in the art, in their laboratory, even when all other factors are the same.

With regard to the use of different inbred strains of mice commonly used to make knout out mice, the specific background of the mice strain can contribute to an observed phenotype. For example, Gerlai et al., (Trends in Neurosci. 19 (5): 177-181, 1996), teaches that most gene targeting is currently carried out in cultured embryonic stem cells derived form the mouse strain '129' (see, p. 178, table 1), once the 129-type ES are introduced into a blastocyst, the surviving chimeric embryos are mated to 'wild-type' non-mutated mice to generate an offspring F1 of heterozygous null-mutant mice. Commonly, the mouse derived from ES cells from mouse strain '129' are mated to wild-type mice from a different strain (e.g., C57BL/6 or BL6) to generate an offspring F1. As a result, comparison of the phenotypes of the heterozygous mutants and wild-type littermates of a second generation (F2), may lead to false positive results because effects of genetic background or genes linked to the knock-out genes, thus the behavioral alterations seen in null-mutant mice may simply be due to the genetic background (see, p. 177 column 2, paragraph 2; p. 179, Fig. 1). The Specification does not disclose the characterization of ES or mating animals to generate the transgenic mice (p. 9, lines 1-13). While Applicant contemplates producing heterozygotes or homozygotes by crossing the chimeric mice with wild-type mice confirming the contribution rate (i.e., chimeric rate) of embryonic stem cells of a produced

chimeric mouse, by using a combination of an embryonic stem cell and the mouse line of an early embryo (p. 9, lines 25-28), the contributions to the observed phenotype of the specific targeting vector introduced into mouse embryonic stem cells (e.g., PJL-5 line) was not assessed, and the specification does not disclose the wild-type control mouse to which one can compared the phenotype. Failure to disclose the identity of the wild-type control mice precludes any evaluation of whether the observed phenotype is within the normal range of variation seen between the inbred mouse strains.

Alternatively, the claims may be interpreted to read on somatic cell gene transfer. The claims, as written, do not specifically convey germline transmission of the transgene and could also be interpreted as just one knock-out cell for the disrupted Bradeion gene in the claimed chimeric mouse generated by injection of embryonic cells into a blastocyst. It would be unpredictable to make and use the claimed chimeric mice comprising merely one cell knock out for the Bradeion gene resulting in reduced or suppressed Bradeion expression wherein said mice has sufficient reduction or suppression of the Bradeion expression so as to exhibit the claimed phenotype. Moreover, it would require further experimentation for the skilled Artisan to try and follow the disclosed instructions to use the claimed chimeric mice with any percentage of chimerism e.g., 10%, 20%, resulting in the claimed functional phenotype. Therefore the guidance provided by the specification amounts to an invitation for one of skilled in the art to determine whether chimeric mice created by somatic cell gene transfer to any cell could be generated and used in accordance with the invention as claimed.

Thus the state of prior Art teaches the challenging issues in gene targeting to generate a null mutation in a mouse resulting in a phenotype that could be clearly interpreted. Hence, one skill

in the Art at the time of the invention was filed could not reasonably predict that the use of a transgenic mouse or a cell, e.g. an isolated ES cell, comprising a disruption in a Bradeion, associated with any malformations characterized by cranial dysplasia, visual disorders, and generalized decreased growth.

The specification teaches in Examples 2 the generation of a targeting vector for disruption of an endogenous Bradeion gene, said targeting vector is introduced into embryonic stem cells (pp. 6-7). In Example 3, the specification discloses the use of stem cell lines 281 and 344 each injected into blastocysts of C57BL/6 mice by microinjection, which are then transferred into the oviducts of pseudopregnant female mice for the embryos to generate and develop into mice (p. 18 bridging to p.19 lines 1-15). Moreover, the specification discloses on Fig. 3A-3D and Fig. 4A-4D chimeric mice generated from embryonic stem cell lines 281 and 344, respectively, exhibiting malformation which includes cranial dysplasia, visual disorders, and generalized decreased growth wherein the chimeric rate is 90% or more and less than 98% (p.3, lines 17-24; p. 19, lines 15-20). The specification teaches at page 19 that chimeric mice derived from mouse embryo wherein a mouse embryonic stem cell was introduced comprising an endogenous Bradeion gene, whose expression was suppressed, have chimeric rates between 90%-98%. Applicants are silent about any observed phenotype for any other percentage of chimerism other than within 90% to 98%. However, the specification provides neither guidance nor working examples for the correlation and association of malformations characterized by cranial dysplasia, visual disorders, and generalized decreased growth and any disruption of the Bradeion gene resulting in reduced expression and exhibiting cranial dysplasia, visual disorders, and generalized decreased growth associated with any percentages of chimerism. Further, the

specification contemplates using the chimeric mice as animals for genetic breeding associated with abnormalities in the cerebral nervous system (p. 19, lines 29-30). However, the specification fails to provide specific examples of any cranial dysplasia, visual disorders, and generalized decreased growth modulated by disruption of Bradeion exhibiting any level of chimerism (e.g., 20%, 60%) and any evidence that the claimed transgenic mice would respond differently that a wild-type mouse in any of these tests, in any useful way.

Prior art teaches the unpredictability in ascribing a particular phenotype to a specific disruption. The mere capability to perform a gene transfer in a mouse may result in a phenotype wherein several genes may participate in the observed phenotype. Additionally, the influence of the environment on the phenotype has to be further considered. Moreover, ignoring the genetic background and contributions of genes linked to the Bradeion gene may lead to misinterpretation of results. Further, the specification does not provide enough guidance for targeting the disruption of the Bradeion gene by any genetic mechanism of gene suppression other than by targeting the Bradeion gene by homologous recombination. As such, and to the extent that the claimed invention is drawn to the make and use of a transgenic mouse encompassing a genus of unspecified variants for phenotypes associated with a Bradeion chimeric mouse exhibiting any percentage of chimerism, using any inbred strains of mice to produce chimeric mice, the as-filed application does not provide sufficient guidance and/or working examples for a skilled artisan to reasonably enable the claimed invention.

In conclusion, the disclosed information from the as-filed application plus the state of the prior art is not deemed sufficient to reasonably convey to one of ordinary skill in the art that the Specification is reasonably enabling for the full breadth of the claim at the time the invention

was made. Such is because chimeric Bradeion mice exhibiting any percentage of chimerism would not reasonably serve as a model for any disease associated with cranial dysplasia, visual disorders, and generalized decreased growth. Because of lack of working examples, insufficient guidance and direction in the specification, the inherent unpredictability in the art, the state of the art and the nature of the invention, one of ordinary skill in the Art to would be required to perform a large amount of experimentation to make and/or use the invention claimed by the Applicant.

Conclusion

No claim is allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding his application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

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